Computational Biology and Bioinformatics
2020; 8(2): 62-71
http://www.sciencepublishinggroup.com/j/cbb
doi: 10.11648/j.cbb.20200802.15
ISSN: 2330-8265 (Print); ISSN: 2330-8281 (Online)

Review Article

Cellular Interactions, Metabolism, Assessment and Control of Aflatoxins: An Update

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To cite this article:

Syeda Mona Hassan, Shahzad Sharif Mughal, Syed Khurram Hassan, Asif Ibrahim, Huma Hassan. Cellular Interactions, Metabolism, Assessment and Control of Aflatoxins: An Update. *Computational Biology and Bioinformatics.* Vol. 8, No. 2, 2020, pp. 62-71.
doi: 10.11648/j.cbb.20200802.15

Received: June 22, 2020; **Accepted**: November 2, 2020; **Published**: November 19, 2020

Abstract: Aspergillus spp., the fungus containing aflatoxin, is commonly spread in nature and contains severely polluted food sources from humans and wildlife, resulting in risks to health and even mortality of species. In plants, such as maize and peanuts, the spores of *Aspergillus paraciticus* and *Aspergillus flavus* can grow on the surface of stigma. The germ tube goes into the developing embryo and mimics pollen germ tubes. Aflatoxins are naturally occurring substances, so it is difficult to remove them completely from products. However, they should be lessened to minimum possible level. There is also a strong need for research into aflatoxins to establish effective methods for their correct identification , quantification and monitoring to ensure public health safety. The chemistry and biosynthesis process of aflatoxins is addressed in a succinct fashion along with their occurrence and the toxic health threats to humans and livestock. This analysis focuses primarily on aflatoxin tools, development, identification and control techniques to ensure food and feed safety. The study is very useful to health-conscious customers and academic authorities in the related fields. In addition , the availability of information on toxicity of aflatoxins would help ensure food safety and solve potential problems for the rising population by reducing the occurrence of outbreaks related to aflatoxins.

Keywords: Aflatoxins, Aspergillus Sp., Secondary Metabolites, Food Contamination, Detoxification

1. Introduction

Aflatoxins are extremely toxic secondary metabolites formed primarily by fungal species like Aspergillus flavus, A. parasiticus. These fungi are mostly infectious to cereal crops such as rice, wheat, walnut, cotton, corn, tree nuts and peanuts [49] and can cause serious health hazards to humans as well asanimalswhich leads to various complicationslike hepatotoxicity, immunotoxicity and teratogenicity [50]. The key aflatoxins are B1, B2, G1 and G2, which induce so much amplification of inflammatory response during the mucous, cutaneous and respiratory cycles in the body (Romani 2004). The quality of food remains one of the most important challenges facing the world today; as a result, several experiments have been performed on clinical, laboratory and epidemiological issues dealing with different aspects of food safety for consumers [2]. Since 1985, a standard for the permittable amount of mycotoxins in food products has been set by the United States Food and Drug Administration (USFDA). A service laboratory for measuring mycotoxin in food and feed kernels has been used by the USDA Grain and Plant Inspection Systems [45]. Furthermore, the World Health Organization (WHO) and Food and Agricultural Organization (FAO) have documented many toxins found in agricultural products. When mycotoxins get into foods, they cannot be

damaged by normal cooking methods. However, there have been developed many recent strategies such as good manufacturing practices (GMP) hazard analysis of critical control points (HACCP) to keep final food products safe and healthy [13, 44]. Furthermore, various physical , chemical and biological techniques are used for detoxifying food in whole or in part and maintaining food quality and consumer health issues. This study presents an overview of aflatoxigenic fungi, chemistry and biosynthesis and their diversity and their health threats for human beings and animals. In addition, diverse physical , chemical and biological methods are now being briefly addressed for food control and management.

1.1. Major Sources of Aflatoxins

The aflatoxins are mostly produced by fungi such as A. parasiticus, A. flavus, and A. nomius (Okun et al., 2015), however they are also synthesized by other species of *Aspergillus* as well as by *Emericella* spp. [51]. There are more than 20 types of known aflatoxins, but the major types are AFB1, AFB2, AFG1 and AFG2 [7], whereas AFM1 and AFM2 are the hydroxylated metabolites of AFB1 and AFB2 [28, 67].

1.2. Biosynthesis of Aflatoxins and Their Metabolites

Biosynthesis of aflatoxins involves polyketide pathways having very complicated process in contribution with more than 25 clusters of genes positioned in a 75 kb region of fungal chromosome [1] and is regulated by the regulatory gene, aflR [66]. It involves many enzymes and genes. However, a large variety of genera and species produce the anthraquinones and some of them produce the xanthones. Final step of oxidative ring cleavage is proceeded by some tropical *Aspergilli* and results in the synthesis of aflatoxin from xanthones. Even among the *Aspergilli*, *Aspergillus flavus* has ability to produce only B group, while *Aspergillus parasiticus* can do more oxidative ring expansion and can produce G group [38].

AFB1 and AFG1 are the unsaturated species, whereas AFB2 and AFG2 are fully saturated species At least 13 aflatoxins have been identified and characterized. The major aflatoxins and their derivatives are as follows: AFB1 and AFB2 are produced by both *A. flavus* and *A. parasiticus* [1]. AFG1 and AFG2 are entirely produced by *A. parasiticus* AFM1 and AFM2 are the hydroxylated derivatives of AFB1 and AFB2 [12].

1.3. Gene Responsible for Aflatoxin Production

Various enzymes and genes are concerned in the biosynthesis of dihydrosterigmatocystin (DHST) and sterigmatocystin (ST), which are the penultimate intermediate of aflatoxins biosynthesis [68]. The aflatoxin biosynthesis gene was named as nor-1, which was first cloned in A. Parasiticus, and is named after the formation of product by this gene during biosynthesis. These genes were named accordingly substrate and the product formed nor-1 (norsolorinic acid [NOR]), norA, norB, avfA (averufin [AVF]), avnA (averanti [AVN]), ver-1 (versicolorin A [VERA]), verA andverB, however these are based on enzyme functions fas-2 (FAS alpha subunit), pksA (PKS), estA (esterase), fas-1 (FAS beta subunit), adhA (alcohol dehydrogenase), vbs (VERB synthase), ordA (oxidoreductase A), dmtA (mt-I; O-methyltransferase I), omtA (O-methyltransferase A), cypA (cytochrome P450 monooxygenase), cypX (cytochrome P450 monooxygenase) and moxY (monooxygenase). At first, the aflatoxin mediated gene was named as afl-2 in A. flavus and apa-2 in A. parasiticus [29].

Therefore, it was referred to as aflR in A. parasiticus, A. flavus, and A. nidulans as is employed as a transcriptional activator. Previous studies revealed that aflA (fas-2), aflB (fas-1), and aflC (pksA) are responsible for the conversion of acetate to NOR [54]. Furthermore, theuvm8 gene was shown to be crucial for NOR biosynthesis as well as aflatoxin synthesis in A. parasiticus. The amino acid of sequence of the gene is similar to that of the beta subunit of FAS, which makes the polyketide backbone during aflatoxin biosynthesis; thus, the uvm8gene was named fas-1 [33].

1.4. Properties of Aflatoxins

Aflatoxins are generally categorized on the basis of their chemical structures into two groups of difuranocoumarins: Difuro coumaro cyclopentenone group: It includes aflatoxin B1 and B2. Difurocoumarolactone group: It includes aflatoxin G1 and G2.

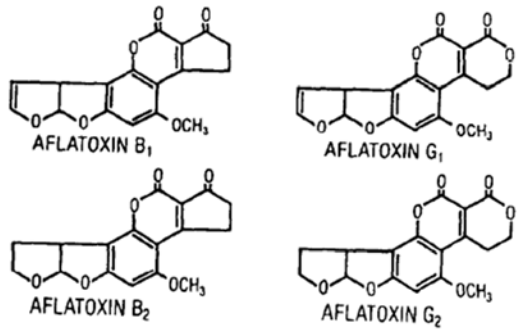

Figure 1. *Structures of aflatoxins [34].*

The order of potency of major aflatoxins is AFB1>AFG1>AFB2>AFG2. The order indicates that Aflatoxin B1 is most toxic, carcinogenic and mutagenic among all the aflatoxins [34]. The aflatoxins show fluorescence strongly in ultraviolet light (365 nm). AFB1 and AFB2 show blue fluorescence whereas AFG1 and AFG2 show green fluorescence [4]. The aflatoxins can be solubilized in moderate polar solvents (e.g. methanol and chloroform) and can also be solubilized in water [37]. Aflatoxins are thermally stable with slight acidic character and can be degraded above 240 °C. They can not be eliminated from food during food processing in industries and cooking or during pasteurization [41]. The lactone ring is present in the aflatoxin molecule which is vulnerable to alkaline treatment. However, the use of acids can be effective to control aflatoxins [37].

1.5. Occurrence of Aflatoxins

Aflatoxins are found in various cereals, oilseeds, spices,

and nuts [38]. The fungi colonize among themselves and synthesize aflatoxins, which contaminate cereals and grains during growth, harvest, transport and storage [39]. Aflatoxins contamination of barley and wheat also occurred due toimproper storage. In milk, aflatoxins is generally at 1–6% of the total content in the feedstuff [1-3]. Aflatoxin can be present in eggs, milk and meat products, as the humans also ingest the aflatoxin-infected food. So, humans are also infected by aflatoxins due to consumption of contaminated food [4-6].

2. Factors Favorizing Aflatoxin Production

Several factors are responsible for aflatoxin production. Aflatoxin production is the outcome of releationship between the host, fungus and the environment. The suitable interactions of these factors are responsible for the type and concentration of aflatoxin produced. Major causes in mold infestation and toxin production are high-temperature stress, humidity (16%) and factors which decrease immunity of host such as insect damage [52, 53].

In plants, such as maize and peanuts, the spores of *Aspergillus paraciticus* and *Aspergillus flavus* can grow on the surface of stigma. The germ tube goes into the developing embryo and mimics pollen germ tubes. So, the myceliums develope an endotrophic interaction which does not damage the healthy plant. But if the plant is stressed than aflatoxins can be produced in the plant tissue during the development in the field and the most common stress is drought. Food can also be contaminated during harvesting but the concentrations of aflatoxins are not as high as in stored food products. Animal feeds, milk and milk products can be contaminated by aflatoxins even in temperate climates where these *Aspergilli* do not generally participate. However, their spores are present in the air all over the world [38] and can be dispersed easily from their natural ecological niches to the vulnerable crops and plants throughout the world [1-3].

2.1. Exposure to Aflatoxins

Humans are mostly infected by direct ingestion of infected crops [7, 8], by eating foods (oil, peanut butter, wine, nut paste, etc) derived from infected commodities [9, 10]. Aflatoxins can also be ingested through infected milk containing AFM1 [11, 12]. Dairy products are also infected by milk aflatoxins (M1 and M2) that mostly occurs in the casein (48.5%), in the milk serum (46.5%) and only a small fraction is present in the fat portion (5%) [13, 14].

Mostly women are involved in agricultural practices such as in weeding, planting and harvesting. Women are also actively involved in cooking food [56]. Therefore, they are mainly influenced by toxic effects of aflatoxins causing health hazards, as they consume these aflatoxins in large quantities with meals in its raw and prepared condition [59].

2.2. Cellular Metabolism

2.2.1. Activation of Aflatoxins

Enzymes catalyze the bioactivation of AFB1 and all enzymes essential for the bioactivation of AFB1 are present in the nuclear envelope of hepatocytes [35]. AFB1 itself not act as a carcinogen but is bioactivated to carcinogenic metabolite in the body [50]. The microsomal mono-oxygenases have mixed function oxygenases (requiring P450, molecular oxygen and NADPH) which oxidizes AFB1 into highly reactive AFB1-8, 9-epoxide form that reacts with DNA to form an N7 guanine adduct and produces chronic effects [34].

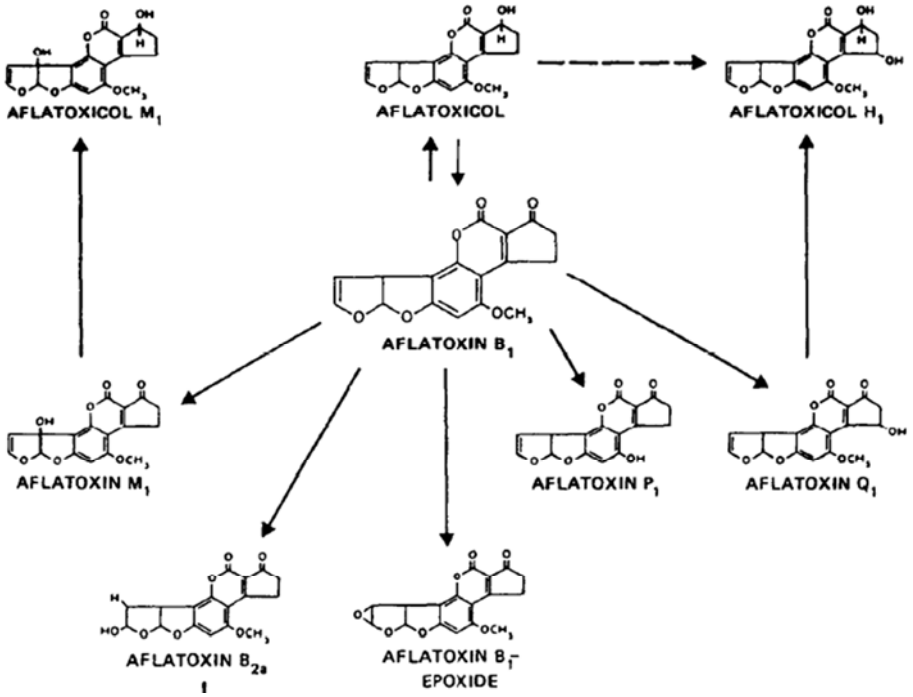

Figure 2. *Metabolism of AFB1* [37].

The mono-oxygenase system in microsomes can transform the aflatoxin B1 into polar molecules such as AFQ1, AFP1 and AFM1 [69]. In these polar molecules, AFQ1 is the major metabolite of AFB1 [64]. AFB1 can be converted to hydroxylated form AFM1 [43] which causes cytotoxicity, DNA damage, chromosomal anomalies, gene mutation, and cell transformation [46]. It was estimated that presence of about 1% to 3% of the AFB1 in animal feed results in the production of AFM1 in milk [34]. Cytochrome P450, present in liver, can activate as well as can detoxify AFB1 [23]. The hepatocytes can also eliminate the AFM1, AFQ1 and AFP1 [64]. Enzyme NADPH-reductase can transform AFB1 to aflatoxicol reversibly. So aflatoxicol can act both as a reservoir and a sink for AFB1 [19].

2.2.2. Inhibition of ATP Generation

AFB1 inhibits the electron transport chain in mitochondria at both ADP-coupled and dDNP-uncoupled levels. It inhibits electron transport chain at the cytochrome oxidase level (site II) and between cytochrome b and c. This inhibition can be reversed by the electron acceptor N, N, N', N'-tetramethylphenylenediamine (TMPD) [6]. AFB1 causes decrease of cellular ATP production. It results in swelling of mitochondria and then sodium, potassium gradient is disturbed within the cell [32].

2.3. Detoxification of Aflatoxins

Detoxification of aflatoxins takes place by forming conjugate of aflatoxins with glucuronic acid, glutathione or sulphate. Conjugation of the glutathione with reactive epoxide is the major detoxification reaction of AFB1 catalysed by glutathione S-transferase. Conjugate of AFB1-glutathione is removed through the bile [19]. As well as, intestinal microflora can potentialy hydrolyse this conjugate. The epoxide hydrolase systems, UDP-glucuronyl-transferase and sulphotransferase can detoxify AFB1-8, 9-epoxide [8]. Many other aflatoxins (AFP1, AFM1, AFG1, and AFH1) form conjugates with sulphate or glucuronide and are excreted out by the urine [19, 20].

2.4. Interaction with Biomolecules

2.4.1. DNA

The activation of aflatoxin B1 and G1 takes place by mixed function oxidase (MFO). The mixed function oxidase is present in the ER of the liver and mediates the formation of the 8, 9 epoxide of AFB1 [44]. The metabolites of aflatoxins act as electrophile and they attack the nucleophilic hetero-atoms such as oxygen and nitrogen in the organic bases of nucleic acids and forms the covalent adducts [64]. There exists a strong correlation between mutagenicity, carcinogenicity and the potential of covalent binding of DNA with aflatoxins and their metabolites. AF-epoxide attacks the DNA on the N^7 position of guanine and AFB1- N^7-guanine is the major adduct. This covalent binding results in mutations which may lead to cancer. Aflatoxin B1 also inhibits the DNA synthesis by attacking the guanine in the DNA which acts as a main target for the attack of activated aflatoxins [31].

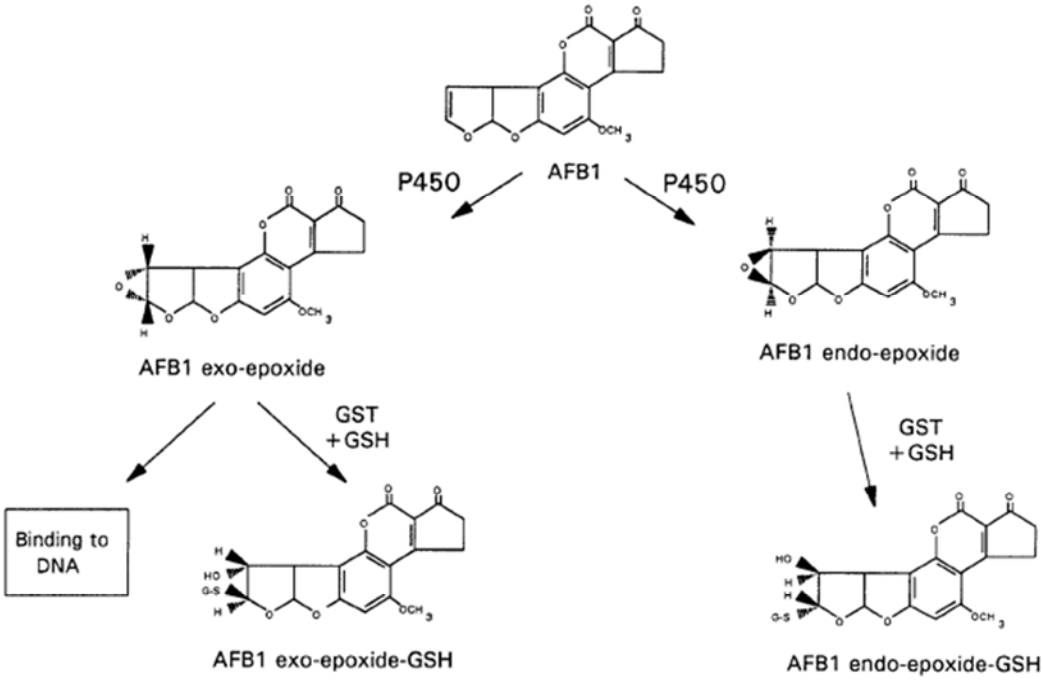

Figure 3. *Glutathione S-transferase (GST) catalyzed conjugation of AFB1 exo- and endo-epoxide with glutathione (GSH)* [60].

2.4.2. RNA

AFB1 inhibit the synthesis of nucleolar RNA by inhibiting the RNA polymerase which is responsible for RNA synthesis and results in the degranulation of endoplasmic reticulum [36].

2.4.3. Proteins

Aflatoxin metabolites act negatively with different cells, which inhibit the protein synthesis [16, 27]. AFB1 inhibits the protein synthesis directly by inactivating the enzymes that are involved in initiation, transcription and translation processes of protein synthesis and indirectly by changing the DNA template activity. They interfere with pyrimidine and purines nucleosides resulting in the inhibition of protein synthesis by the formation of adducts with DNA, RNA and proteins [6]. The specific reversible non-covalent and non specific-irreversible covalent binding with aflatoxins can change the activities and structure of proteins [50].

2.4.4. Carbohydrate and Lipid Metabolism

Aflatoxin metabolites react with different cell, which results in the inhibition of lipid and carbohydrate metabolism and decreased liver function [16, 28]. AFB1 inhibits the glyconeogenesis process by reducing glycogen synthase and transglycosylase enzyme functions which catalyses the rearrangement and elongation of glycogen molecules. AFB1 also reduces the activity of phosphoglucomutase which reversibly changes glucose-6-phosphate into glucose-1-phosphate and also decreases the hepatic glycogen by activating glucose-6-phosphate oxidation [6].

AFB1 causes lipid deposition in the liver. It is not because of increased lipid biosynthesis but is due to impaired lipid transport. Lipid deposition in the liver is due to less oxidation of fat as mitochondria are being damaged. AFB1 also affect lipid absorption and degradation [37, 47].

3. Specific Effects of Aflatoxins

3.1. Immune Response

Aflatoxin supresses the immune functions by inhibiting the lymphocytic activity, innate immune responses and antibody production. AFB1 exposure lowers the level of immunoglobulin which leads to reduction of acquired immunity. Exposure of AFB1 to lymphocytes causes reduction in the response of lipopolysacaride. AFB1 also decreases the T-lymphocytes and natural killer cell. AFB1 also inhibits the phagocytic ability of macrophages [11].

3.2. Hormonal Effects

Steroid hormones bind non-covalently and specifically to membranes in target cells and cytoplasmic receptor proteins to regulate cellular functions. Then hormone-receptor complexes are transfered to the nucleus and then selective gene transcription (mRNA) is stimulated by binding with the chromatin acceptor sites. AFB1 binds covalently with DNA specifically at the guanine, so decrease the acceptor sites of nucleus for binding of hormone receptor complexes and result in less activity of the hormone. AFB1 also reduces the acceptor sites of nucleus for the glucocorticoid cytosol receptor complex in the liver [30].

3.3. Mutagenic and Teratogenic Effects

AFB1 and its epoxide are the strongest mutagen. AFB1 causes chromosomal disorders such as chromosomal fragments, chromatid and DNA breakage in animal and plant [18]. Several mycotoxins, including AFB1 are teratogenic. Aflatoxins are strong inhibitors of protein synthesis and may cause damage to feotal differentiation and primordial development [61].

3.4. Carcinogenic Effects

In 1993, WHO International Agency for Research on Cancer (IARC) categorized the aflatoxins as Group 1 carcinogens [34]. Chemicals causing carcinogenesis may be categorized as initiators and promoters [23]. AFB1, AFG1, AFM1 can act both as initiator and promoter. Cancer cells are formed by normal cells in two steps. During initiating step, biochemical lesions are produced in RNA and specifically DNA and mutations occur in adducts. These cells are cancerous, but under favourable conditions, they undergo proliferation. The transformed cells become malignant and start propagating independent of normal cellular regulatory procedure. AFB1 binds covalently to mitochondrial DNA with more affinity than with nuclear DNA. Lesions in mitochondrial DNA are persistent. So, mitochondrial translation and transcription may be prevented by these lesions. This leads to the neoplastic conversion of the cells [30].

Aflatoxins are strong hepatic carcinogens and mutagens. Mutation occurs at codon 249 in the tumor suppressor gene TP53 by aflatoxin which induces carcinogenesis. In this mutation, mutant allele is R249S and the mutant protein is p. R249S. This type of mutation causes up to 75% of hepatocellular carcinoma [21].

The p53 which is a tumour-suppressor gene not only involved in the mutations of gene which results in tumor, but also regulates transcription and translation of other genes [38]. The liver cancer is by the modification of the structure of liver cells DNA [34].

3.5. Health Aspects

Clinical, epidemiological and experimental studies showed that AFB1 may results in carcinogenesis [10]. The toxic effects of aflatoxins can be classified into two groups such as chronic toxicity and acute toxicity [65].

Aflatoxin B1 itself is not toxic but is metabolised to toxic forms in the animal body. Its metabolism determines whether it will cause chronic or acute toxicity [38]. The main target organ for toxicity, mutagenicity and carcinogenicity is the liver [43]. The M1 and M2 are derivatives of aflatoxin B1 and B2. They get deposited in tissues and can also be excreted out through milk [41].

AFB1 increased the rate of heamolysis because of lipid peroxidation in plasma membrane, cell lysis and permeability alterations. Aflatoxins cause oxidative damage and lipid peroxidation of liver and other organs. These processes are initiaied by hydroxyl radicals [64].

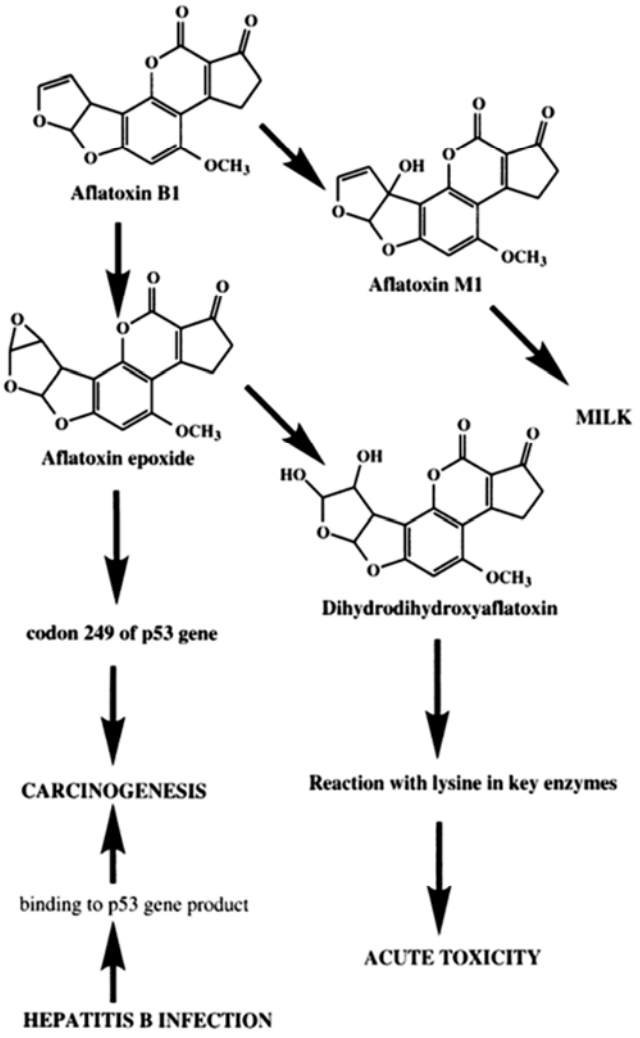

Figure 4. *Metabolites of aflatoxin B1 involved in toxicosis [34].*

3.6. US FDA Regulations

Aflatoxins are important not only because of toxic effect to public health but also cause economical losses [1]. Annualy, they cause 923 million US Dollar of economic loss in the grain industry of United States [41]. FAO also estimated that mycotoxins contaminate more than 25% of the world agricultural production, annually [41, 52]. FDA has developed acceptable levels of aflatoxins for humans and animals which are less than 20 and less than 100 ppb, respectively[4]. Occurance of aflatoxins in poultry and animal feedstuffs is relatively common in many countries and cause great economic losses. So, the protection of food and feed from the aflatoxins is a vital requirement [58].

3.7. Analysis of Aflatoxins

Several cultural methods are used for the detection of aflatoxins. Such as the blue fluorescence detects aflatoxins in the medium and fluorescence is enhanced by fluorescence enhancer such as cyclodextrin and iodine. Aflatoxin producing colonies turned into plum-red when exposed to ammonium hydroxide vapors. These cultural methods have advantages as they are inexpensive and no major equipment is required [59].

A number of conventional analytical techniques have also been used for the estimation of aflatoxins in food and feed samples such as: thin layer, gas or liquid chromatography, spectrophotometry, spectrofluorometry [6], HPLC, HPTLC, immuno chemical assays like ELISA, immuno chromatographic strip and flow system coupled capillary electrophoresis [62].

TLC is the simplest and cost effective method. Mostly, sillica gel plate, developing tank with mobile phase and long wavelength UV radiation are required for seperation and identification of aflatoxins. The mobile phases are diethyl ether-methanol-water (96:3:1), chloroform-acetone (9:1) and anhydrous diethyl ether [26]. TLC plate method is effective for qualitative as well as for quatitative estimation of aflatoxins, which relates to the visualization of distinctive fluorescent spots and their intensities on TLC plates [67].

Anokwuru et al. determined the aflatoxin contents in peanut kernels during storage. Microbiological analysis was carried out by growing them on culture medium "Sabouraud's Dextrose Agar" and then chemically analysed by TLC method. Aflatoxins were present in 35 samples, whereas *Aspergillus flavus* was seperated from 26 samples. AFB1 present in the samples was varied from 17.57 to 404.00 µg/Kg kernel [5].

ELISA is a suitable technique for the analysis of aflatoxins in a number of samples in very short time. This method can be used for the screening of total aflatoxins. It was determined that 13 samples of nuts, out of 142, were contaminated above the maximum tolerated level (15 µg/kg) [57].

Ozaslan et al. determined the aflatoxin contents in *P. vera* by ELISA technique. AFB1 was present in 16 samples with concentration of more than 2 ppb. HPLC technique was also used for the determination of aflatoxins [42].

HPLC with fluorescent detector has become the most accepted method used for aflatoxins determination [40]. Aflatoxins in nuts were determined by HPLC. Aflatoxins were extracted from sample by methanol: water: n-hexane (80:20:20). Immunoaffinity column was used for clean-up and mobile phase was composed of acetonitrile: methanol: water (17:29:54). The sample was eluted from the column at a flow rate of 1 ml/min and detected at at λ_{ex} 365 nm and λ_{em} 435 nm. HPLC showed high efficiency by recovering the aflatoxins from 85.6 to 112.3% [57].

Mushtaq et al. investigated the concentration of aflatoxins in 125 samples of processed food by using RP-HPLC with fluorescent detector. The aflatoxins were extracted by aqueous acetonitrile and immunoaffinity column was used for clean-up. It was found that 38% of processed food samples were contaminated with aflatoxins. The AFB1, AFB2, AFG1 and AFG2 were present in processed food samples [40].

Villa at al. determined the AFB1 in breakfast cereals by using HPLC with fluorescence detector. Sample was extracted by methanolic-aqueous solvent followed by clean-up with immunoaffinity column. Sample was eluted from column by using mobile phase of water: aceonitrile: methanol (20: 4: 3) and detected at excitation and emission wavelengths of 365 and 425 nm, respectively. Recovery of AFB1 was 78%, while the detection limit was 0.02 ppb.

In recent years, vibrational spectroscopy based on NIR spectroscopy is a fast method used to detect the aflatoxins. NIR spectra are based on comparatively broad and weak overtone. Fourier transform technique in the NIR region (4000–12,000 cm^{-1}) improves the spectral reproducibility and wave number precision. FT-NIR is a fast and easy instrument with high precision, accuracy and potential to carry out nondestructive analyses. It detects the physical and chemical sample parameters from a single spectrum, which enables several components to determine simultaneously [62].

4. Control Strategies

Mycotoxins control strategies should be economically reliable and should meet the criteria listed by the FAO/UNEP/WHO on mycotoxins. According to this criteria, control strategy should: (I) destroy or inactivate the toxin (II) end products should not be toxic or carcinogenic (III) destroy the mycelia and fungal spores that they should not reproduce, (IV) nutritive value of the product should not be destroyed (V) should not change important properties of the product [41].

Aflatoxins are naturally occurring substances, so it is difficult to remove them completely from products. However, they should be lessened to minimum possible level [39]. Many cheap and environmentally sustainable methods are used to minimize the aflatoxin contamination. These methods can be applied as pre or post-harvest treatment of crops. Some methods give early identification and separation of contaminated kernels of peanuts or corn. Genetically resistant crop strains, proper irrigation and biopesticide treatment in which a non-aflatoxigenic strain of *Aspergillus* can be used to remove the toxic strains [59]. Adsorption methods are cost effective and can efficiently remove the aflatoxins and their secondary metabolites from organic and aqueous solutions [41]. Chemisorbent compounds are also used to reduce the deleterious effects of aflatoxins [48]. Such as activated charcoal and Hydrated sodium calcium aluminosilicate adsorb AFB1 and prevents the absorbtion of aflatoxins in the digestive tract and is beneficient in reducing the harmful effect of aflatoxins [16].

Cytosolic glutathione S-transferases detoxify the reactive AFB1-epoxide by forming conjugate and modulate the AFB1-DNA binding. Hepatic glutathione S-transferases with antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene, ethoxyquin and dithiolthiones are effective for the prevention of AFB1-DNA binding and AFB1 hepatocarcinogenesis [25]. Chlorophyllin can act as mutagen against a broad range of compounds including aflatoxins [15].

Non-nutritional components are present in food with biological activity and health beneficial effect. Coffee-specific diterpenes cafestol and kahweol are biologically active components [65]. They act as chemoprotective agents against various toxicants and procarcinogens such as AFB1. This activity involves two mechanisms *(1)* Reduction of cytochrome P450 responsible for the conversion of AFB1 to genotoxic AFB1-8, 9-epoxide (AFBO). *(2)* Induction of glutathione transferase (GST) involved in the inactivation of AFBO [9]. Vitamin A is a vital bio-antioxidant. Vitamin A (Retinol) abstracts the free radical proton from the α-carbon of polyene chain atoms and scavenges reactive oxygen species. Vitamin A reacts with bioactive AFB1 and AFG1 and forms inactive AFB2 and AFG2 [64].

Different organic acids such as acetic acid, propionic acid are used as effective mold inhibitor in animal feed. Propionic acid prevents the aflatoxin production by preventing the growth of *A. flavus* and *A. paraciticus*. Sodium bisulphite and sodium hydroxide are used as degrading agents of aflatoxins. They act as antifungal agents to inhibit the aflatoxin production [16, 17].

Safara et al. investigated the detoxification of aflatoxins in rice by citric acid. He treated the 275 contaminated samples of rice by citric acid and examined them by HPLC. It was analysed that 97.22% aflatoxins were degradated in rice by the treatment with 1N citric acid [55].

Ozaslan et al. determined the efficacy of various organic acids to prevent mould growth and aflatoxin contamination in fenugreek seeds. Among all organic acids, benzoic acid was found to be most effective. 0.4% benzoic acid completely detoxified AFB2 and AFG1 at the concentration of 0.4% but 0.5% of benzoic acid could detoxify AFB1 only up to 95.70%. However, mould growth was totally inhibited at this concentration [42].

Albores et al. evaluated the effect of sodium, potassium, and calcium hydroxide at three different concentrations (10, 20, and 30 g kg^{-1}) on cocoa liquors contaminated with aflatoxins. The higher concentrations (20 and 30 g kg^{-1}) of all chemicals showed almost same effectiveness in aflatoxin degradation (up to 98%). Alkaline treatment of cocoa liquors enhanced their physicochemical properties and also improved their sanitary quality by reducing the aflatoxin concentration [3].

Gowda et al. investigated the antifungal potential of various chemicals and herbal compounds at different concentrations. Then these antifungal compounds were applied in feeds to test their efficacy to inhibit fungal growth and aflatoxin production in feeds. All the chemical and herbal compounds significantly ($P<0.01$) reduced the mould growth and aflatoxin production. Among all the chemical compounds sodium propionate, propionic acid, ammonia and benzoic acid showed highest anti-fungal activity, followed by urea and citric acid. Among all the herbal compounds, clove oil showed best activity followed by turmeric, garlic and onion [22].

Instead of the pure chemicals, plants and microorganisms and their active metabolites can also detoxify aflatoxin. A lot of natural sources such as plants, herbs and essential oils are relatively safe. Essential oil of *S. hortensis* has potent antifungal activity against aflatoxigenic *A. parasiticus*. It has strong antifungal, antioxidant, anti-inflamatory and antibacterial activities [1].

Velazhahan et al. evaluated the efficacy of aqueous extracts from seeds and leaves of different medicinal plants to inhibit AFG1 production. Among the various medicinal plant extracts, the seed extract of *T. ammi* showed the maximum detoxification of AFG1 up to 65%. *T. ammi* extract can be a biologically safe way to protect poultry feeds from aflatoxins [63].

Anokwuru et al. analysed the ability of aqueous ethanolic extract of *A. indicia* bark to reduce hepatotoxicity and damage of blood cells induced by dietary aflatoxins. Feed treated with different concentrations of extracts of *A. indicia* were given to mice. It was evaluated that *A. indicia* extract (50 mg/kg) prevented the hepatotoxic effect induced by dietary aflatoxin.

Rajani et al. investigated the efficacy of aqueous extracts of various medicinal plants including *Punica granatum, Cassia alata, Datura stramonium, Polyanthia longifolia* and *Annona squamosa* against *Aspergillus parasiticus* (NCIM 898). The extracts were applied in different concentration of 5%, 10%, 15%, 20% and 25%. It was evaluated that *Polyanthia longofolia* extract showed significant inhibition (73%) of *A. parasiticus* (NCIM 898) and aflatoxin production [48].

Martins et al. evaluated the antifungal and antimycotoxigenic activities of juca (*Libidibia ferrea*) and guarana (*Paullinia cupana*) extracts. The extracts were applied at different concentrations (1.08, 1.62 and 3.24%). It was found that both fruit extracts showed significant inhibition of aflatoxins, produced by *A. parasiticus*, as compared to control [35].

Hassan et al. also investigated the anti-aflatoxigenic activity of *Punica granatum* and *Ziziphus jujuba* leaves in *Aspergillus parasiticus* inoculated broiler feed. The commercial broiler feed was treated with plant leaves powder (5%, 10% and 15% w/w) and stored for the period of six months at different temperature and moisture conditions. Aflatoxins (AFTs-B1, B2, G1, G2) concentrations were determined at the end of each month by HPLC method and compared with control. *P. granatum* leaves inhibited AFTs production up to 100% up to four months of storage, whereas *Z. Jujuba* leaves exhibited AFTs production up to 3 months. The *P. granatum* and *Z. Jujuba* leaves AFTs inhibition in feed inoculated with A. parasiticus revealed that these plants are the potential source of antifungal activity [24].

5. Conclusion

Aflatoxins are a major source of disease outbreaks due to a lack of knowledge and consumption of contaminated food and feed worldwide. Extreme levels of aflatoxins in food of undeveloped countries are of major concern. Several effective physical, chemical, biological, and genetic engineering techniques have been employed for the mitigation, effective control and management of aflatoxins in food. But, developing fungal resistant and insect resistant hybrids/crops to combat pre-harvest infections and their outcome is a major issue of concern. Post-harvest treatments to remove aflatoxins such as alkalization, ammonization, and heat or gamma radiation are not generally used by farmers. However, some of plants have the ability to degrade and reduce the aflatoxin contamination in different types of agricultural products. Therefore, methods of using these organisms to reduce aflatoxin are currently being focused on. Moreover, application of genetic recombination in *A. flavus* and other species is being investigated for its potential to mitigate aflatoxins to ensure the safety and quality of food.

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